Residual Impurities in Biopharmaceutical Products

Process-related impurities or residual impurities are formed at any time during upstream or downstream processes. They are compounds that are present at very low concentrations in complex biomanufactured products and can vary from large proteins to small chemicals. Therefore, the proper detection and evaluation of residuals requires extensive knowledge of biotherapeutics manufacturing as well as solid expertise in current analytical methodology. This article reviews the most common types of residual impurities and identifies appropriate monitoring methods for a successful adaptive control strategy.

Introduction

Biopharmaceuticals represent a wide range of therapeutic drugs manufactured in living cells or organisms. The characterisation of these products is particularly difficult because biopharmaceuticals are composed of several different structures, more commonly referred to as variants. Variants of the biopharmaceutical product presenting different safety and/or efficacy profiles are defined as product-related impurities. On the other hand, process-related impurities or residual impurities are not structurally related to the intended biopharmaceutical drug and can occur at any time during the upstream or downstream process. This makes their detection and evaluation particularly difficult, requiring extensive knowledge of the biopharmaceutical drug manufacturing process.

Different impurity types can form at various stages during the manufacturing process. It should be noted that adventitious viruses, endotoxins and mycoplasma are considered as contaminants, not impurities, and therefore will not be discussed in this article.

Types of Impurities:

- The first impurity type to be considered are upstream impurities, which are split into two categories, cell-substrate derived and cell culture-derived impurities. Examples of cell-substrate derived impurities are host cell proteins (HCPs), DNA residues or virus-like particles. Examples of cell culture-derived impurities include antibiotics, inducers, antifoam or media components, and virus inactivating agents.

- The second type are downstream impurities, are common components such as purification reagents (chromatographic solvents, buffers), column and tubing leachates, or metals.

- The third important type of residuals are raw or ancillary material related impurities. Compendial monographs often provide useful standardised tests and specifications for assessing the purity, presence and concentration of raw and ancillary materials.

- The fourth and final category of impurities are formed during the storage phase of the product and may interact with the biotherapeutic itself. Hence, monitoring of key stability indicators such as critical Quality Attributes (or cQAs) of the drug substance is an essential step of the drug development pathway. The appearance of non-product related impurities during storage should always be part of the Control Strategy.

Biopharmaceutical Characteristics

The concept of a well characterised biopharmaceutical product is usually acknowledged as the physico-chemical and biological characterisation of the drug substance itself. However, the identification and quantification of both product-related and process- related impurities is also an integral part of the definition of a well characterised product.

One of the goals of process control and monitoring during the manufacturing process is to ensure the safety and efficacy of the final product as well as to minimise any unwanted effects. Ensuring that a biopharmaceutical is safe can only be done by filing a complete and well-defined product profile. For well-known biologic modalities such as Monoclonal Antibodies (MAbs), proteins expressed in Chinese Hamster Ovarian (CHO) or Escherichia Coli cells, the permissible levels of the most common impurities (such as protein A, aggregates, or HCPs...) is well documented. Even the removal of these common impurities is part of established adaptive control strategies.

For other, less-common classes of biologic drugs, including Antibody Drug Conjugates (ADCs), Gene Therapy products, Oligonucleotides (DNA, RNA), Cell Therapy products, or Enzymes, in depth studies are required to establish efficient monitoring of process related impurities. This starts with defining the Quality Target Product Profile (QTPP), followed by identifying which of the possible process related impurities will be classified as cQAs.

Residual impurities

Examples of commonly encountered residual impurities are listed in the table below, together with suggested monitoring methods. The impurities can be segregated into two major groups, chemical-based and biological-based. Most biological-based impurities such as residual DNA, host cell proteins, or fetal bovine serum, require specific monitoring methods such as PCR or Immunoassays. On the other hand, online LC-MS could address most of the commonly encountered chemical-based impurities. This is why real-time, direct analysis will play an increasingly important role in control of the manufacturing process of biopharmaceuticals. Because of its versatility, LC-MS is a perfect example of a real-time, direct analysis technique that can become an essential tool for In Process Control (IPC) monitoring. This will ultimately facilitate the implementation of Quality by Design (QbD) criteria in the manufacturing of

biotherapeutics, which will in turn result in significant time and cost savings.

However, the analysis of aggregates or particles in solution is particularly challenging, mainly due to their broad size range which can vary by up to six orders of magnitude[1] from soluble dimers to large, insoluble aggregates. Once identified, these impurities should be clearly classified as either process related or product related, to allow for a better control strategy. Since no analytical technique is available to cover a six order of magnitude size range, a combination of analytical methods is needed to accurately characterise this type of impurity. Finally, the presence of trace metals can be monitored with Inductively Coupled Plasma (ICP) Spectroscopy.

	IMPURITY TYPES	MONITORING METHOD
Upstream	**Cell substrate derived:**	
	Host cell protein	Immunoassays, MS
	Host residual DNA and RNA	PCR
	Virus like particle	Nanoparticle tracking analysis, SEC-MALS
	Cell culture derived:	
	Antibiotic (Amoxicillin, Chloramphenicol, Kanamycin...)	HPLC, MS
	Antifoam (Tween, Pluronic acid, PPG...)	HPLC, MS
	Biological Ingredients (Insulin, FBS...)	HPLC, Immunoassays
	Chelating agents such EDTA	HPLC, MS
	Inducers such IPTG	HPLC, MS
	Metal ions	ICP
	Process enhancing agents (DTT, Glutathione...)	HPLC, MS
	Selective agent such Methotrexate	HPLC, MS
	Solubilizers (Guanidine, Urea...)	HPLC, MS
Downstream		
	Buffer components (Citrate, Glycine, Imidazole, Sucrose, Tris...)	HPLC, MS
	Leachates (columns, tubing)	HPLC, MS
	Protein A	HPLC, Immunoassays
	Residual solvents (Acetonitrile, DMF, DMSO...)	HPLC, MS
	Visible particles	Appearance
	Subvisible particles	Light obscuration, Liquid particle counter, Micro-flow imaging, Nanoparticle Tracking Analysis (NTA), Direct Light Scattering (DLS), Size Exclusion Chromatography-Multi Angle Light Scattering (SEC-MALS), Transmission Electron Microscopy (TEM)

Table 1: Examples of common families of residual impurities with suggested monitoring methods

Control Strategy Plan

In order to detect and quantify process related impurities, sensitive methods have to be established and validated. This provides the only means to effectively develop an efficient process with an associated control strategy plan, to guarantee the elimination of impurities, or at least their reduction to an acceptable safety level.[2]

The first step in assuring the purity of a biopharmaceutical product is to prepare a strategic plan, such as the one below:

1. Identify the nature and source of each process related impurity
2. Assess the risk for patient safety of each residual impurity. The risk should be weighed against the probability of occurrence of each impurity
3. Develop a method sensitive enough to quantitate the residual impurities at various steps of the manufacturing process (for the Drug Substance (DS) and the Drug Product (DP)).
4. Develop a process capable of eliminating or at least reducing any process related impurities to an acceptable level
5. Set the acceptance criteria for the residual impurities in the DS and DP

The regular improvement of existing methodologies or the development of new ones, helps in continuously lowering the sensitivity levels for detection and measurement of process related impurities. These technological advancements, especially in Mass Spectrometry, make it possible to monitor various impurities and product quality attributes in parallel with the development of a Multiple Attribute Monitoring (MAM) concept.[3] Moreover, if connected to a production stream, MAM may allow real-time, in-process control so that production parameters can be instantly adjusted.

Examples of In Process Control (IPC)

During the second chromatographic step of the downstream processing of a Monoclonal Antibody (Mab), an aliquot of the eluent fraction from ion exchange chromatography was collected for residuals and product quality attribute analyses. LC-MS monitoring data for the residual concentration of a polyethylene glycol antifoam and the level of oxidation of a specific Methionine residue are given below as two examples of IPC.

The figure below shows 3 traces: the buffer blank, the buffer blank spiked at 0.5ug/mL of the antifoam and the sample. Four parent ions were selected across the distribution of the antifoam mass spectrometric signals to be fragmented in the collision cell. A common fragment ion was monitored for each parent. The antifoam level in the sample was calculated at 0.13ug/mL.

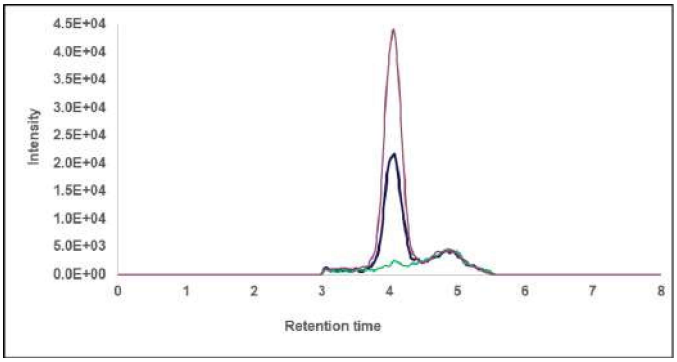

Figure1: LC-MS/MS chromatogram showing the TIC (total ion chromatogram) obtained from the sum of four transitions specific to the antifoam agent used. Top trace: standard sample spiked at 0.5ug/mL, middle trace collected sample, bottom trace blank buffer eluate.

The MS chromatogram below shows the MS profiles of a native tryptic peptide generated by enzymatic digestion of the MAb (top trace) and its oxidised variant (lower trace). The ratio of peak areas allows the oxidation level of a specific Methionine residue within the Mab sequence to be estimated at 4.9%. To evaluate Methionine oxidation, it is important that the oxidised peptide produced by the enzymatic treatment of the parent drug does not coelute with its corresponding native parent peptide, since oxidation can occur in the detector. Moreover, the sample preparation protocol should be carefully developed so as to avoid the occurrence of oxidation.

The increasing use of disposable manufacturing equipment coupled with the recent development of continuous or semi-

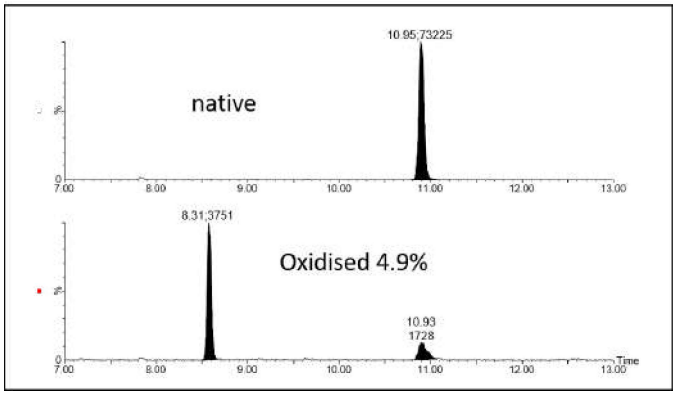

Figure 2: LC-MS chromatogram showing the SIR traces of the native Methionine containing peptide (top trace) and its oxidized variant (bottom trace). Respective peak areas are integrated.

continuous downstream processes makes the implementation of IPC with MAM methodologies much easier. When applied early, these developments allow a better understanding of the manufacturing operation and product attributes. This greatly facilitates the execution of a QbD approach[4] for the production process, resulting in consistently shorter processes, an improved control strategy, and a reduction in the number of QC tests needed for batch release.

Conclusion

The identification and monitoring of residual impurities is an essential step in the development of biotherapeutic drugs. With new modalities of biologics emerging, such as oligonucleotides, RNAs, next-generation peptides, antibody drug conjugates, cell & gen therapies and others, new potential impurities will continue to emerge. These novel impurities will require new methods of monitoring and sensitive analytical technologies will need to be developed. MAM and therefore MS-based methodologies are extremely valuable, as they can provide "real-time", in-process control monitoring of a wide range of impurity types. The recent development of continuous downstream processes can become the ideal testing ground for direct, real-time analysis, facilitating the adoption of QbD principles for biopharmaceutical manufacturing.

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